

LEUCINOSTATIN D, A NOVEL  
PEPTIDE ANTIBIOTIC FROM  
*PAECILOMYCES MARQUANDII*

Sir:

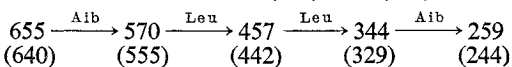
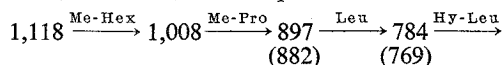
Recently, we reported the isolation and structure elucidation of two peptide antibiotics, **1** and **2**, produced by submerged cultures of *Paecilomyces marquandii* (Masse) Hughes.<sup>1,2</sup> In independent studies, Japanese groups have proposed identical structures for leucinostatins A (**1**) and B (**2**).<sup>3,4</sup> Isolated from culture filtrates of *Paecilomyces lilacinus* A-257. In the very early stages of our isolation work it became evident that the bulky residue left from the benzene extraction of the culture broth might contain additional metabolites related to **1** and **2**, the major biologically active components of the extract. Subsequent repeated flash chromatography of the residue has, in fact, led to the isolation of a new peptide metabolite, C<sub>57</sub>H<sub>103</sub>N<sub>11</sub>O<sub>11</sub>, for which we propose the name leucinostatin D.<sup>5</sup> The present communication describes its characterization and spectral data establishing its structure **3**.

The new metabolite [white crystals from EtOAc; mp 184~185°; fast atom bombardment mass spectrometry (FAB-MS) *m/z* 1,118 (M<sup>+</sup>); UV λ<sub>max</sub><sup>EtOH</sup> nm (ε) 202 (27,930), 220 (sh, 18,830); IR ν<sub>max</sub><sup>CHCl<sub>3</sub></sup> cm<sup>-1</sup> 3310 (NH), 1650 (amide CO)] gave NMR spectra very similar to those obtained for **1** and **2**.<sup>1,2</sup> The low field region of the <sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>) revealed the presence of nine amidic NH groups attesting to the peptidic nature of **3**, and a pair of olefinic protons in *trans* relationship, a feature also common to **1** and **2**. The similarity between **3** and **1** is further emphasized by the observation of a six-proton-intensity singlet at 3.12 ppm due to N(CH<sub>3</sub>)<sub>2</sub> while the occurrence of 18 (1 primary, 11 secondary and 6 tertiary) CCH<sub>3</sub> signals between 1.53 and 0.85 ppm suggested a structural pattern reminiscent of both leucinostatins A and B. Information regarding the distinctive structural features of **3** could be gleaned from a comparative analysis of the <sup>13</sup>C NMR data (100 MHz, CDCl<sub>3</sub>). The inventory (C<sub>57</sub>), multiplicity (13 quaternary, 15 CH, 9 CH<sub>2</sub> and 20 CH<sub>3</sub>) and chemical shift values of the <sup>13</sup>C resonances gave the elemental composition C<sub>57</sub>H<sub>103</sub>N<sub>11</sub>O<sub>11</sub> and disclosed that the signals assigned to 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid residue in

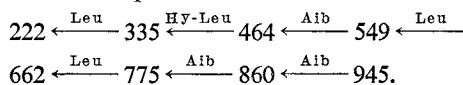
**1** and **2** [176.55 (s, C-1), 54.92 (d, C-2), 34.73 (t, C-3), 25.84 (d, C-4), 45.52 (t, C-5), 63.96 (d, C-6), 50.76 (t, C-7), 211.53 (s, C-8), 36.73 (t, C-9), 19.93 (q, 4-CH<sub>3</sub>), 7.63 (q, C-10) ppm] were replaced by a set of resonances attributable to an additional Leu unit [176.28 (s, C-1), 55.06 (d, C-2), 39.21 (t, C-3), 25.23 (d, C-4), 22.80 (q, C-5), 21.77 (q, C-6) ppm] in the new metabolite.

The structural conclusions inferred from spectral data have received full support from chemical transformations. In accordance with the proposed basic peptidic nature, metabolite **3** gave negative ninhydrin and positive Dragendorff and Reindel Hoppe reactions. Acid hydrolysis (6 N HCl, 120°C, 10 hours in sealed tube) monitored by amino acid analysis yielded the following components (in the order of their retention times; found mol in parentheses): 1 β-hydroxy-leucine (Hy-Leu) (0.89), 1 γ-methylproline (Me-Pro) (1.05), 3 α-aminoisobutyric acid (Aib) (3.19), 3 leucines (3.25) and 1 β-alanine (0.97). Extraction of the hydrolysate with ether afforded an oily residue which, after purification, was identified as a 1:1 mixture of 4-methyl-4-ethylbutyrolactone (**4**) and (*S,E*)-4-methyl-2-hexenoic acid (Me-Hex) (**5**). The extracted hydrolysate, after careful neutralization and subsequent extraction with ether for 4 days, left a residue which, after treatment with dilute HCl and evaporation under reduced pressure, gave (*S*)-1-*N,N*-dimethyl-2-aminopropane·2HCl (**6**) crystalline from EtOH. The structure and stereochemistry of the degradation products **4**~**6** followed from spectral (UV, IR, [α]<sub>D</sub>, <sup>1</sup>H and <sup>13</sup>C NMR) observations and from comparison with authentic samples obtained from the acid hydrolysis of **1** and **2**.

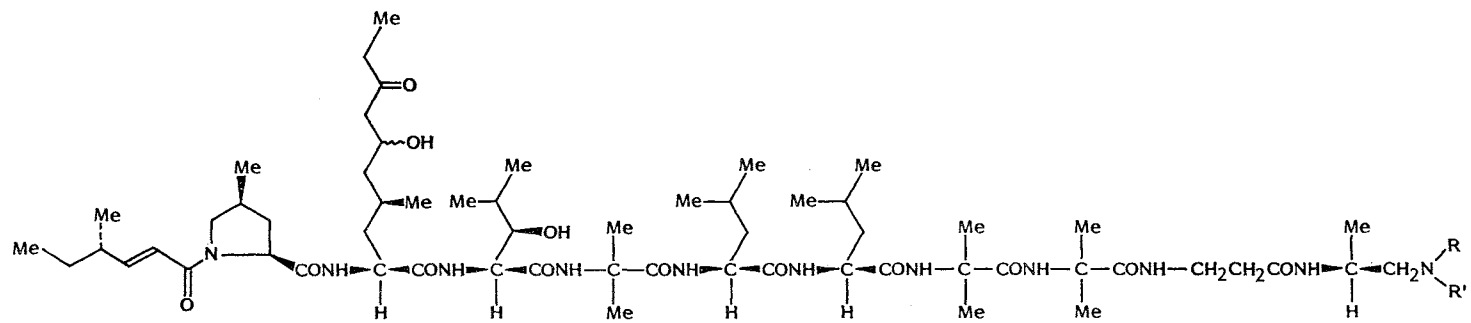
Conclusive evidence for structure **3** was provided by FAB mass spectral analysis. In a similar manner to leucinostatin A, the constitution of the new metabolite readily followed from the +CA and +CALK sequence ions:



and, *via*-cleavage of the peptide linkages, also from the sequence:

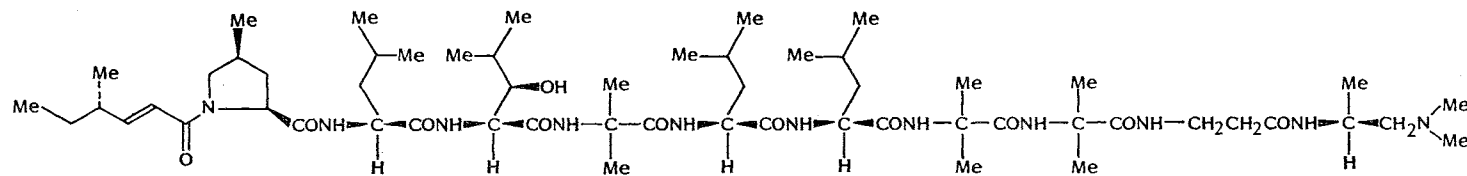


Leucinostatin D shows biological activity

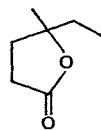


**1** R = R' = CH<sub>3</sub>

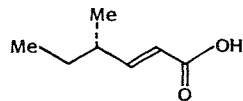
**2** R = H R' = CH<sub>3</sub>



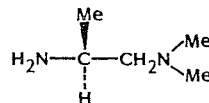
**3**



**4**



**5**



**6**

Table 1. Biological activity of leucinostatin D<sup>a</sup> (MIC, µg/ml).

<i>Bacillus subtilis</i> ICI	12
<i>Micrococcus luteus</i> ISS	4
<i>Streptococcus pneumoniae</i>	12
<i>S. haemolyticus</i>	12
<i>Staphylococcus aureus</i> Smith	6
<i>S. aureus</i> 39/2 <sup>b</sup>	6
<i>Escherichia coli</i> 0147/ISS	100
<i>Pseudomonas aeruginosa</i>	100
<i>Salmonella typhi</i>	100
<i>Shigella sonnei</i> B68	100
<i>Proteus vulgaris</i>	100
<i>Candida albicans</i> CBS 562	10
<i>C. utilis</i> 93/ISS	25
<i>Torulopsis famata</i> 4077/ISS	2
<i>Cryptococcus neoformans</i> 4710/ISS	4
<i>Microsporium canis</i> 2155/ISS	4
<i>Trichophyton mentagrophytes</i> I	6
<i>T. mentagrophytes</i> F	1
<i>T. tonsurans</i> L	1
<i>T. verrucosum</i> 1466/ISS	2
<i>Tricosporon undulatum</i> 6805/ISS	10

<sup>a</sup> Minimum inhibitory concentrations were obtained by the dilution method. Media for bacteria consisted of nutrient broth, for fungi SABOURAUD's broth.

<sup>b</sup> Penicillin-resistant.

against Gram-positive bacteria and several fungi (see Table 1). Of particular interest is the activity exhibited against some *Staphylococcus* strains that are known to be resistant against benzylpenicillin or macrolide antibiotics.<sup>5)</sup> Phytotoxicity tests on tomato cuttings proved positive at 2 µg/ml concentration (irreversible withering of the cuttings within 72 hours)<sup>6)</sup> and *in vitro* assays on cytotoxic activity resulted in the following ID<sub>50</sub> values (ng/ml): 850 (HeLa), 0.95 (KB) and 1.00 (P388/S).

After completion of this work we learned from Prof. K. L. RINEHART, Jr., that, by using FAB-MS techniques, his group has arrived to structure 3 for a leucinostatin component isolated from culture filtrates of *P. lilacinus* A-257.<sup>7)</sup>

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Financial support of the Italian National Research Council (C.N.R.) under project scheme "Progetto-finalizzato Chimica Fine e Secondaria" is greatly acknowledged.

CARLO ROSSI  
LORENZO TUTTOBELLO  
MAURIZIO RICCI

Istituto Chimica Farmaceutica,  
Università degli Studi di Perugia,  
I-06100 Perugia, Italy

CARLO G. CASINOVÌ

Laboratorio Chimica del Farmaco,  
Istituto Superiore di Sanità,  
Viale Regina Elena, 299,  
I-00161 Rome, Italy

LAJOS RADICS\*

NMR Laboratory,  
Central Research Institute  
of Chemistry,  
P.O. Box 17, H-1525 Budapest,  
Hungary

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